Arnaldoa 13(2): 270 - 275
2006

ISSN: 1815-8242

Cytogenetic studies in *Schinus* species (Anacardiaceae)

Estudios citogenéticos en especies de *Schinus* (Anacardiaceae)

María Laura Las Peñas, Gabriel Bernardello
Instituto Multidisciplinario de Biología Vegetal (UNC-CONICET), C.C. 495, 5000 Córdoba, ARGENTINA.
lauralp@imbiv.unc.edu.ar
Pedro E. Steibel *and* ***Héctor O. Troiani***
Facultad de Agronomía, Universidad Nacional de La Pampa, C.C. 300, 6300 Santa Rosa, La Pampa, ARGENTINA.

Resumen

Se estudiaron por primera vez los números cromosómicos somáticos y se aplicaron técnicas de bandeo CMA_3/DAPI a nueve especies de *Schinus* del sur de América del Sur: *S. areira*, *S. fasciculatus*, *S. johnstonii*, *S. longifolius*, *S. o'donellii*, *S. patagonicus*, *S. praecox*, *S. roigii*, y *Schinus* sp. (una nueva especie aún no descripta a fin a *S. praecox*). Todas las especies presentaron 2n = 28, resultados que sugieren un número básico de x = 7. Los cromosomas fueron de tamaño pequeño, midiendo desde 0,6 a 1,9 μm. El largo total del genoma haploide en todas las especies estudiadas fue en promedio de 1,1 μm. Cinco especies (*S. areira*, *S. fasciculatus*, *S. longifolius*, *S. o'donellii*, *S. roigii*) presentaron un par cromosómico de mayor tamaño con un satélite en el brazo corto. Se realizaron bandeos cromosómicos con CMA_3/DAPI en todas las especies, excepto en *S. longifolius* por escasez de material. Con esta técnica, aplicada aquí por primera vez en Anacardiaceae, se observó un par cromosómico con una banda CMA^+/$DAPI^-$ terminal, asociado con la región organizadora nucleolar. En ninguna especie se observaron bandas CMA^-/$DAPI^+$.

Palabras clave: números cromosómicos somáticos, bandeos CMA_3/DAPI, *Schinus*, Anacardiaceae.

Abstract

We here present results on the somatic chromosome numbers and CMA_3/DAPI banding of nine southern South American *Schinus* species: *S. areira*, *S. fasciculatus*, *S. johnstonii*, *S. longifolius*, *S. o'donellii*, *S. patagonicus*, *S. praecox*, *S. roigii*, and *Schinus* sp. (a new species to be described akin to *S. praecox*). All species were examined cytologically for the first time and showed 2n = 28, a result suggesting that x = 7 is the basic number for the genus. Chromosomes were small in size, ranging from 0.6 to 1.9 μm, being quite homogeneous among all the species analyzed. The average chromosome length for all the species studied was 1.1 μm in average. Five species (*S. areira*, *S. fasciculatus*, *S. longifolius*, *S. o'donellii*, *S. roigii*) had a larger chromosome pair that bore a small terminal satellite in the short arm, whereas the remaining species showed homogeneous sized chromosomes with a pair with terminal satellites in the short arm. CMA_3/DAPI double staining was performed in all species, except *S. longifolius* for which we had not enough material. This technique, applied for the first time for Anacardiaceae, revealed one chromosome pair with a CMA^+/$DAPI^-$ terminal band, associated with a nucleolar chromosome. No bands CMA^-/$DAPI^+$ were detected in any species.

Key words: somatic chromosome number, CMA_3/DAPI banding, *Schinus*, Anacardiaceae.

Introduction

The genus *Schinus* L. belongs to Rhoeae, the largest tribe of Anacardiaceae (Mitchell & Mori, 1987), and comprises around 30 South American species (Barkley, 1957; Muñoz, 2000). Its range of distribution includes Peru, Bolivia, Chile, Paraguay, Brazil, Argentina, and Uruguay. Its center of diversification is thought to be northern Argentina (Barkley, 1957), a region where 22 species grow spontaneously. Some *Schinus* species have become established in tropical and warm regions of the World, where they were introduced (Parodi, 1980). *S. areira* L. (known as "Peruvian pepper tree", "aguaribay", "aroeira vermelha", "corneíba", etc.) is widely cultivated for the abundant production of coral-red odorific fruits which contain essential oils and piperine and are used as condiment (Brücher, 1989).

These plants are shrubs or trees with alternate leaves and are typical because of its fruit: a small drupe with a thin exocarp and a resinous mesocarp that adheres to a bony endocarp (Barkley, 1957). Concerning its reproduction, they are polygamo-dioecious (Muñoz, 2000).

In spite of the systematic, evolutionary, and cytological importance of the chromosome numbers, the genus *Schinus* has been scarcely studied from this point of view. As few as two species have been reported so far: *S. molle* L. (Schnack & Covas, 1947; Copeland, 1959; Oginuma *et al.*, 1993), and *S. polygamus* (Cav.) Cabrera (Schnack & Covas, 1947). This lack of knowledge may be due to the small chromosome size of the members of the family (e.g., Cáceres & Avila Todesco, 1987; Vogt & Aparicio, 1999; Fasihi Harandi & Ghaffari, 2001), a circumstance that also prevents building karyotypes. For the Anacardiaceae as a whole, the original basic chromosome number is x = 7, with most of the evolution proceeding at the tetraploid level (Raven, 1975). The available chromosome reports show a wide range of numbers: 2n = 30, 40, 28, 32 and 20 in decreasing order of frequency (e.g., Fedorov, 1969; Goldblatt, 1985; Goldblatt & Johnson, 2000).

Chromosome location and cytochemical characterization of constitutive heterochromatin by fluorescence staining procedures has been applied with success in several plant families (e.g., Guerra, 2000; Souza & Benko-Iseppon, 2004), but in Anacardiaceae it has not been attempted.

Upon this background, in this contribution we present results on the somatic chromosome numbers and CMA_3/DAPI banding of nine *Schinus* species that grow naturally in southern South America.

Material and methods

All species analyzed were collected in the wild, in Argentina. Vouchers are kept in the herbarium of the Facultad de Agronomía - Universidad Nacional de La Pampa (SRFA) (Table 1).

Seeds were soaked for 24 h in running water and then put in Petri dishes on moist filter paper and stored at 30°C. Root tips were fixed in a 3:1 ethanol:acetic acid mixture, after pretreatment in saturated solution of 8-hydroxyquinoline for 3 h; they were stored in 70% ethanol at 4-6°C until required. For slide preparation, root tips were hydrolyzed with HCl 1 N for 30 min at room temperature and then washed, stained with Feulgen for 2 h, and squashed in a drop of 2% acetic carmine. Permanent mounts were made following the method of Bowen (1956). The number of individuals and cells examined are included in Table 1.

CMA_3/DAPI double staining was performed in all the species studied, except *S. longifolius* for which we had few seeds available (Table 1). The meristems were washed twice in distilled water (10 min each), digested with a 2% cellulase-20% pectinase solution (30 min) and squashed in 45% acetic acid. Only one root tip was used in each slide. After coverslip removal in liquid nitrogen, the slides were aged for three days, stained with CMA_3 (1 h), counterstained with DAPI (30 min), and finally mounted in McIlvaine's buffer-glycerol v/v 1:1. In all species, three individuals and ten cells were observed.

Metaphases were photographed with a phase contrast optic Axiophot microscope and pictures were taken with a Leica DFC300FX camera.

Results and Discussion

All taxa are examined cytologically for the first time and show the somatic chromosome number 2n = 28 (Table 1, Fig. 1, 2). The chromosomes are small in size, ranging from 0.6 to 1.9 μm, being quite homogeneous among the species analyzed. The average chromosome length for the species studied is 1.1 μm ($\pm$ 0.25). It is interesting to note that in five of them (*S. areira*, *S. fasciculatus*, *S. longifolius*, *S. o'donellii*, and *S. roigii*) there is a larger chromosome pair that bears a small satellite in the short arm (Fig. 1, 2). Regarding the chromosome type, all of them seem to be either metacentric or submetacentric.

The double staining with CMA_3/ DAPI revealed one chromosome pair with a CMA^+/ $DAPI^-$ terminal

band (Fig. 2), in the species examined with this technique. This band is associated with a nucleolar chromosome. On the other hand, no bands CMA-/DAPI+ were detected in any species.

As observed in *Schinus*, woody angiosperms mostly have small chromosomes with little differences in size between related species or genera (e.g., Stebbins, 1971; Ehrendorfer, 1976). Thus, it seems that the diversification in the genus *Schinus* has been associatde with a few chromosome rearrangements visible with conventional and CMA/DAPI staining, i.e. large duplication, pericentric inversions, and reciprocal translocation of segments of unequal size. In this sense, cumulative small and cryptic structural changes may have occurred and have played a role in its evolution, as reported in other angiosperms (Bernardello & Anderson, 1990; Acosta *et al.*, 2005).

The somatic chromosome number 2n = 28 was reported for the only two previously studied species: *S. polygamous* and *S. molle* (Schnack & Covas, 1947; Oginuma *et al.*, 1993, respectively); however, Copeland (1959) also informed 2n = 30 for the latter species as part of an embryological study, a report that has to be confirmed. These results suggest that x = 7 is the basic number for the genus *Schinus*, in agreement with the original basic chromosome number proposed for the Anacardiaceae by Raven (1975). In addition, 2n = 28 was reported for other genera of the family, such as *Lannea*, *Pistacia*, and *Schinopsis* (e.g., Fedorov, 1969; Go'dblatt, 1985; Goldblatt & Johnson, 2000). However, Anacardiaceae is heterogeneous in their chromosome numbers, with these numbers reported: 2n = 30, 40, 28, 32 and 20, in decreasing order of frequency (e.g., Fedorov, 1969; Goldblatt, 1985; Goldblatt & Johnson, 2000). Thus, with the available data no trends within the five recognized tribes of the family (Mitchell & Mori, 1987) can be drawn.

Polyploidy was a relevant evolutionary mechanism for the family, as it was for vascular plants as a whole (Bretagnolle *et al.*, 1998). According to Wendel (2000), approximately 70% of angiosperms species could be considered polyploidy, as happens with most Anacardiaceae. The majority of its chromosome reports indicate 2n = 30, 40, and 28, i.e., the evolution of the family was at the tetraploid level. Nevertheless, it should be mentioned that the family has been scarcely examined cytologically: as few as 14% of its ca. 875 species are known in their chromosome numbers (e.g., Fedorov, 1969; Goldblatt, 1985; Goldblatt & Johnson, 2000).

Both Gadek *et al.* (1996) and Pell & Urbatsch (2000) used the chloroplast gene rbcL to investigate, in a molecular phylogenetic context, all families included in the order Sapindales (sensu APG 2003). According to these results, Burseraceae and Sapindaceae are the most closely related families to Anacardiaceae. The scarce chromosome data available indicate that Burseraceae has 2n= 22-26, and Sapindaceae show 2n = 22, 24, 28 and 30 (Fedorov, 1969; Goldblatt, 1985; Goldblatt & Johnson, 2000). Although the numbers are variable, in the three families polyploidy is frequent and the basic number goes from x= 7 to x= 9. The same is valid for the other families of the order: Meliaceae (2n = 28, 42 and 52) and Rutaceae (2n = 18 and 20) e.g., (Fedorov, 1969; Goldblatt, 1985; Goldblatt & Johnson, 2000).

Regarding the application of fluorochrome banding on Anacardiaceae, it should be mentioned that we found no previous reports. Published data on tropical woody plants show a great variation in the heterochromatin distribution (Morawetz, 1986); on the contrary, our data on *Schinus* reveal a homogenous distribution. After the double staining with CMA_3/DAPI, one CMA^+/$DAPI^-$ band could be observed in all studied species, probably corresponding to a NOR-associated heterochromatin, as found in other species from other families (Morawetz, 1986; Guerra, 2000). This evidence suggests that *Schinus* species are relatively poor in heterochromatin, a typical feature of plants with small chromosomes (Guerra, 2000; Gitaí *et al.*, 2005).

Other families of Sapindales have been studied with CMA/DAPI. As the *Schinus* species here studied, in *Urvillea* (Sapindaceae) there is few

heterochromatine as CMA+ bands (Urdampilleta *et al.*, 2006). On the other hand, in *Citrus* (Rutaceae) there are many different heterochromatic bands that are useful to differentiate species (Guerra, 1993; Cornelio *et al.*, 2003). These results show that more cytological data on these interesting plants are badly needed to fully understand the chromosomal evolution within *Schinus*.

Acknowledgements

The authors thank Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), SECYT (Universidad Nacional de Córdoba, Argentina), Agencia Córdoba Ciencia S.E., and Facultad de Agronomía (Universidad Nacional de La Pampa) for financial support.

Literature Cited

Acosta, M. C., G. Bernardello, M. Guerra & E. A. Moscone. 2005. Karyotype analysis in several South American species of *Solanum* and *Lycianthes rantonnei* (Solanaceae). Taxon 54: 713-723.

Angiosperm Phylogeny Group II [APG]. 2003. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. Bot. J. Linn. Soc. 141: 399-436.

Barkley, F. A. 1957. A study of *Schinus* L. Lilloa 28: 5-110.

Bernardello, L. M. & G. J. Anderson. 1990. Karyotypic studies in *Solanum* Section Basarthrum (Solanaceae). Amer. J. Bot. 77: 420-431.

Bowen, C. 1956. Freezing by liquid carbon dioxide in making slides permanent. Stain Technol. 31: 90.

Bretagnolle, F., F. Felber, F. G. Calame & P. Küpfler. 1998. La polyploïde ches les plantes. Bot. Helvetica 108: 5-35.

Brücher, H. 1989. Usefulplants of Neotropical origin. Springer-Verlag, Berlin Heidelberg, New York.

Cáceres, M. E. & P. Avila Todesco. 1987. Cromosomas mitóticos de *Schinopsis haenkeana* y *Loxopterigium grisebachii* (Anacardiaceae). Kurtziana 19: 47-51.

Copeland, H. F. 1959. The reproductive structures of *Schinus molle* (Anacardiaceae). Madroño 15: 14-25.

Cornelio, M. T. M. N., A. R. Figueiroa, K. G. B. Santos, R. Carvalho, W. S. Soares Filho, & M. Guerra. 2003. Chromosomal relationships among cultivars of *Citrus reticulata* Blanco, its hybrids and related species. Plant Syst. Evol. 240: 149-161.

Ehrendorfer, F. 1976. Evolutionary significance of chromosomal differentiation patterns in Gymnosperms and primitive Angiosperms., In C. B. Beck [ed.], Origin and early evolution of angiosperms. Columbia University Press, New York and London.

Fasihi Harandi, O. & M. Ghaffari. 2001. Chromosome studies on Pistachio (*Pistacia vera* L.) from Iran. Options Médit. 56: 35-40.

Fedorov, A. A. (ed.). 1969. Chromosome numbers of flowering plants. Reprinted 1974 by O. Koeltz Sci. Publ., Koeningstein, Germany.

Gadek, P. A., E. S. Fernando, C. J. Quinn, S. B. Hoot, T. Terrazas & M. W. Chase. 1996. Sapindales: Molecular delimitation and infraordinal groups. Amer. J. Bot. 83: 802-811.

Gitaí, J., R. Horres, & A. M. Benko-Iseeppon. 2005. Chromosomal features and evolution of Bromeliaceae. Plant Syst. Evol. 253: 65-80.

Goldblatt, P. 1985. Index to Plant Chromosome numbers 1982-1983. Monogr. Syst. Bot. Missouri Bot. Gard. 13. Missouri Botanical Garden.

Goldblatt, P. & E.D. Johnson. 2000. Index to Plant Chromosome numbers 1998-1999. Monogr. Syst. Bot. Missouri Bot. Gard. 43. Missouri Botanical Garden.

Guerra, M. 1993. Cytogenetics of Rutaceae. V. High chromosomal variability in *Citrus* species revealed by CMA/DAPI staining. Heredity 71: 234-241.

Guerra, M. 2000. Patterns of heterochromatin distribution in plant chromosomes. Genet. Mol. Biol. 23: 1029-1041.

Mitchell, J. D. & S. A. Mori. 1987. The Cashew and its relatives (*Anacardium*: Anacardiaceae). Mem. New York Bot. Gard. 42: 1-76.

Morawetz, W. 1986. Remarks on karyological differentiation patterns in tropical woody plants. Plant Syst. Evol. 152: 49-100.

Muñoz, J. D. 2000. Anacardiaceae. Flora Fanerogámica Argentina, fasc. 65: 1-28 CONICET, Córdoba, Argentina.

Oginuma, K., A. Kato, H. Tobe, S. G. Mathenge, & F. D. Juma. 1993. Chromosomes of some woody plants in Kenya. Acta Phytotax. Geobot. 44: 53-58.

Parodi, L. R. 1980. Enciclopedia argentina de agricultura y jardinería. 3ª ed. Ed. Acme, Buenos Aires.

Pell, S. K. & L. Urbatsch. 2000. Evaluation of evolutionary relationships in Anacardiaceae using matK sequence data. Amer. J. Bot. 87 (suppl.): 149.

Raven, P. H. 1975. The bases of angiosperm phylogeny: cytology. Ann. Missouri Bot. Gard. 62: 724-764.

Schnack, B. & G. Covas. 1947. Estudios cariológicos en antófitas. Haumania 1: 32-41.

Souza, M. & A. N. Benko-Iseeppon. 2004. Cytogenetics and chromosome banding patterns in Caesalpinioideae and Papilionioideae species of Pará, Amazonas, Brazil. Bot. J. Linn. Soc. 144: 181-191.

Stebbins, G. L. 1971. Chromosomal evolution in higher plants. E. Arnold, London.

Urdampilleta, J. D., M. S. Ferrucci, J. M. D. Torezan, & L. L. Vanzela. 2006. Karyotype relationships among four South American species of *Urvillea* (Sapindaceae; Paullinieae). Plant Syst. Evol. 258: 85-95.

Vogt, R. & A. Aparicio. 1999. Chromosome numbers of plants collected during Iter Mediterraneum IV in Cyprus. Bocconea 11: 117-169.

Wendel, J. F. 2000. Genome evolution in polyploids. Plant Molec. Biol. 42: 225-249.

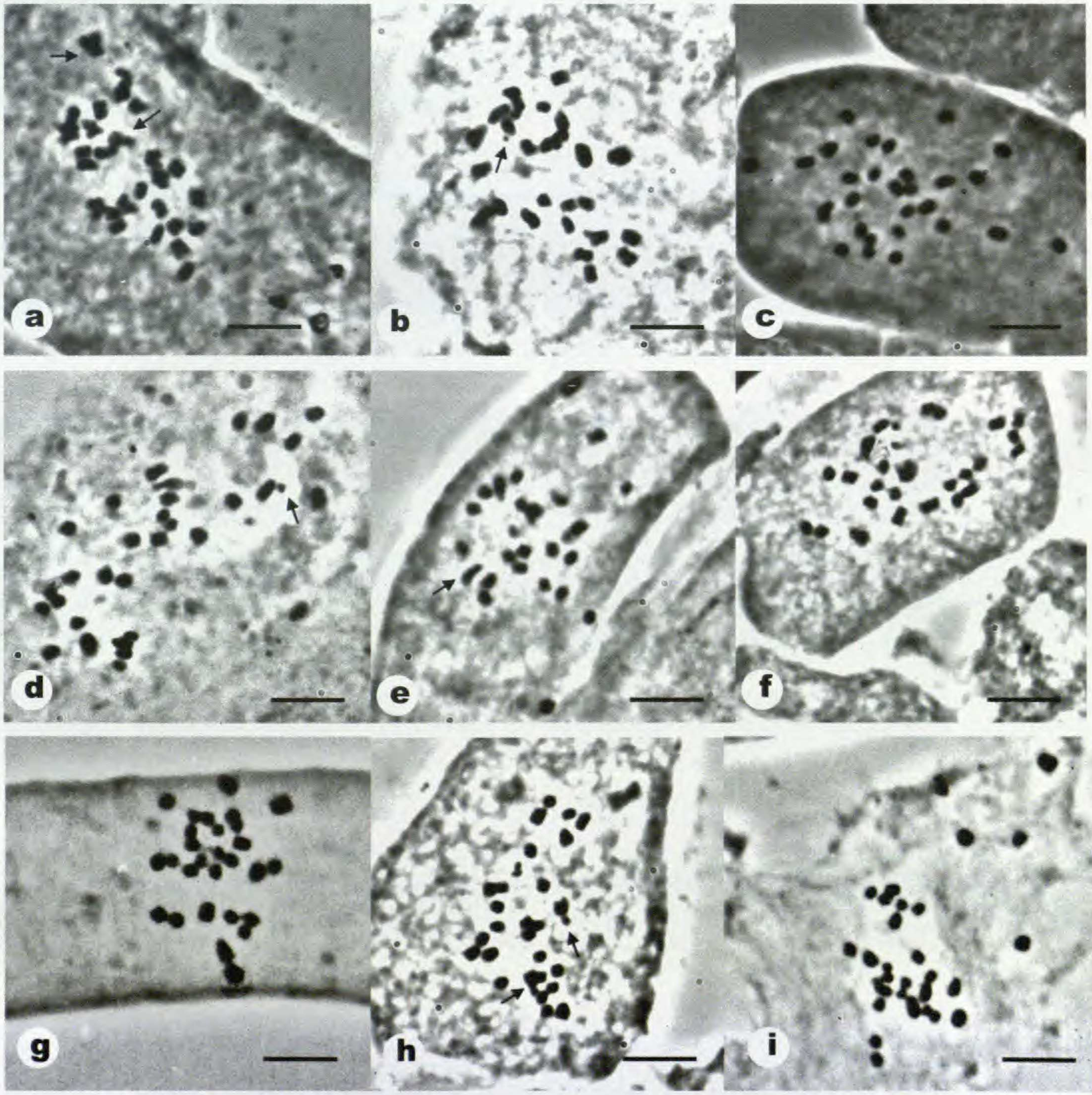

Fig. 1: Metaphase chromosomes of *Schinus* species; **a.** *S. areira*; **b.** *S. fasciculatus*; **c.** *S. johnstonii*; **d.** *S. longifolius*; **e.** *S. o'donellii*; **f.** *S. patagonicus*; **g.** *S. praecox*; **h.** *S. roigii*; **i.** *S.* sp. Arrows indicate the pair of larger chromosomes. Bar corresponds to 5 μm.

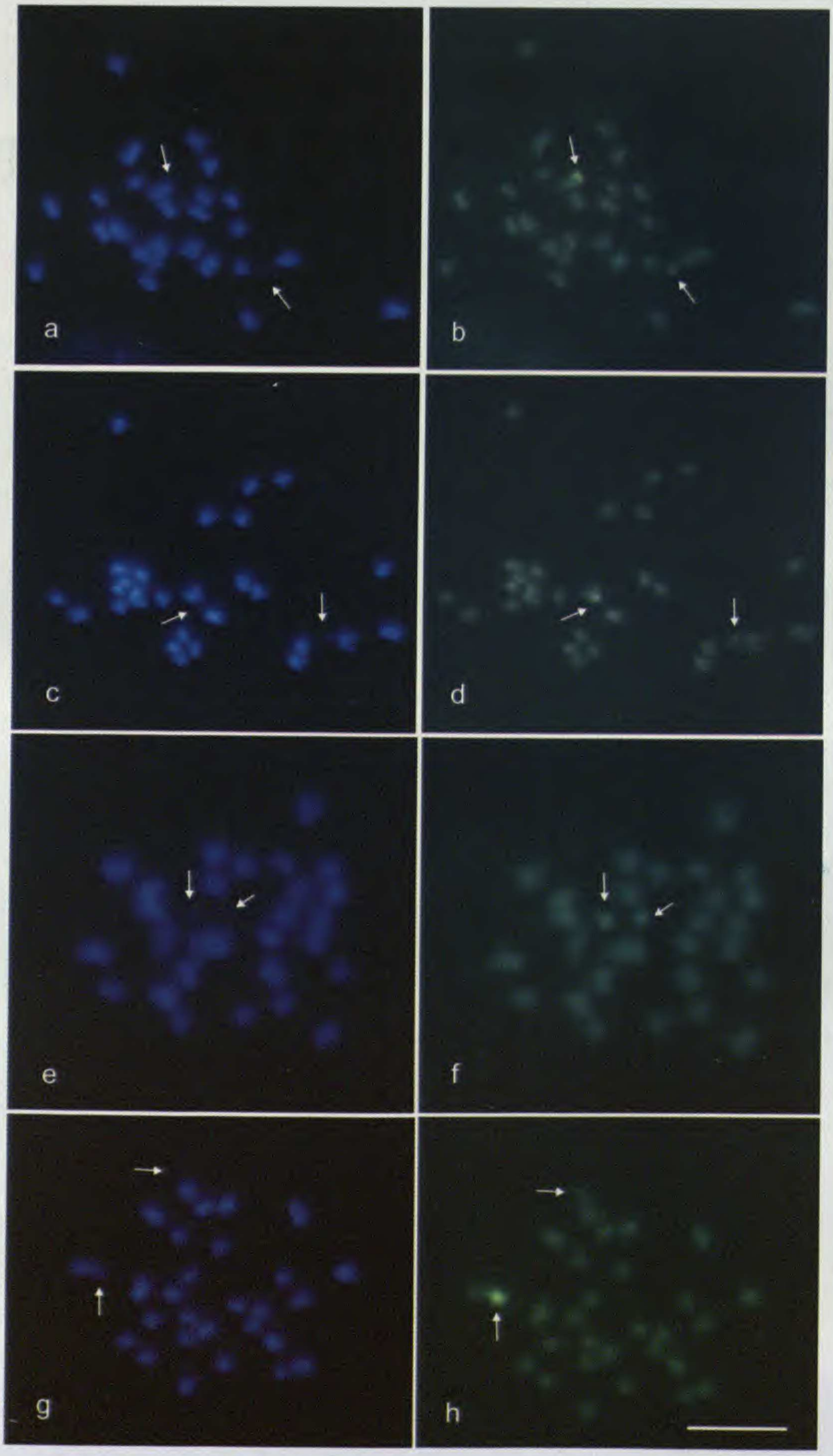

Fig. 2: Chromosomes of *Schinus* species after sequential staining with the fluorochromes DAPI (a, c, e, g) and CMA_3 (b, d, f, h). a-b. *S. areira*; c-d *S. fasciculatus*; e-f *S. praecox*; g-h *S. johnstonii*. Arrows mark $CMA^+/DAPI^-$ NOR-associated heterochromatin. Bar corresponds to 5 μm.